Chelation of Cadmium

by Ole Andersen*

The toxicity of cadmium is determined by chelation reactions: *in vivo*, Cd^{2+} exists exclusively in coordination complexes with biological ligands, or with administered chelating agents.

The Cd^{2+} ion has some soft character, but it is not a typical soft ion. It has a high degree of polarizability, and its complexes with soft ligands have predominantly covalent bond characteristics. Cd^{2+} forms the most stable complexes with soft donor atoms ($S >> N > 0$). The coordination stereochemistry of Cd^{2+} is unusually varied, including coordination numbers from 2 to 8. Even though the Cd^{2+} ion is a d^{10} ion, disturbed coordination geometries are often seen. Generally, the stability of complexes increases with the number of coordination groups contributed by the ligand; consequently, complexes of Cd^{2+} with polydentate ligands containing SH groups are very stable. Cd^{2+} in metallothionein (MT) is coordinated with 4 thiolate groups, and the log stability constant is estimated to 25.5.

Complexes between Cd^{2+} and low molecular weight monodentate or bidentate ligands, e.g., free amino acids (LMW–Cd), seem to exist very briefly, and Cd^{2+} is rapidly bound to high molecular weight proteins, mainly serum albumin. These complexes (HMW–Cd) are rapidly scavenged from blood, mainly by the liver, and Cd^{2+} is redistributed to MT. After about 1 day the Cd-MT complex (MT–Cd) almost exclusively accounts for the total retained dose of Cd^{2+}, independent of the route of exposure. MT–Cd is slowly transferred to and accumulated in kidney cortex. The acute toxicity and interorgan distribution of parenterally administered Cd^{2+} are strongly influenced by preceding MT induction, or decreased capacity for MT synthesis; however, the gastrointestinal (GI) uptake of Cd^{2+} seems unaffected by preceding MT induction resulting in considerable capacity for Cd^{2+} chelation in intestinal mucosa, and this finding indicates that endogenous MT is not involved in Cd^{2+} absorption. The toxicity of parenterally administered Cd^{2+} is strongly enhanced when administered as complexes with NTA or STPP, but it is much decreased when administered as a complex with EDTA. In chronic oral exposure the toxicity and GI uptake of Cd^{2+} is not changed when Cd^{2+} is administered as a complex with the detergent formula chelating agents NTA, EDTA and STPP. The uptake of Cd^{2+} from ligated intestine *in vivo* was not affected by administration of Cd^{2+} as complexes with CYS or GSH, but significantly reduced by complexation with EDTA or BAL. The acute toxicity of orally administered Cd^{2+} is reduced when Cd^{2+} is administered as a complex with EDTA. The renal toxicity and deposition of Cd^{2+} is markedly increased when Cd^{2+} is parenterally administered as MT–Cd. Orally administered Cd–MT is taken up by intestinal mucosa and transferred to kidneys, where MT is broken down. Perfusion of intestinal loops *in situ* with MT–Cd induces necrosis of intestinal mucosa, not observed with Cd^{2+} alone.

During acute treatment of Cd^{2+} poisoning the superior agents seem to be the polyamino-polycarboxylates, especially DTPA, which increases renal excretion. The time lapse between Cd intoxication and antidote administration is critical due to the rapid intracellular accumulation of Cd^{2+}, mainly in liver. During chronic Cd^{2+} exposure, BAL significantly increases biliary excretion of Cd^{2+}, and part of the mobilized Cd^{2+} is chelated out of MT.

Introduction

The element cadmium (Cd) was discovered in 1817. Since then Cd has gained increasing technological importance, and the annual world

smelter production of Cd has increased from 10,000 short tons in 1958 to 18,780 in 1974. Cd is mainly obtained as a by-product from smelting zinc ore, where Cd often is present as an impurity (0.2–0.3%). Cd metal and compounds of Cd are used for electroplating (50% of world production), Cd-bearing alloys, Cd–Ni batteries, pigments, stabilizer in PVC plastics, and control rods in

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nuclear fission reactors. Human exposure to Cd has taken place in industries producing and utilizing Cd, but serious exposures have also occurred in the zinc industry, in operations (mainly welding) on Cd-plated items, in scrap remelting, and due to environmental pollution. Today Cd is ubiquitous in the biosphere. Due to the great concern regarding present and future human health aspects of Cd pollution, a number of reviews of chemical, environmental and biological effects of Cd have appeared (1–8).

Cd is very toxic to mammals both during acute and chronic exposure. In biological systems, soluble Cd^{2+} inevitably exists as coordination complexes with biological ligands, and the toxicity of Cd^{2+} is beyond doubt mediated through chelation reactions at the target. Although the exact mechanism of Cd toxicity has not been established in any organism, the modifying effect of metallothionein on Cd toxicity is widely recognized (9,10). Recent reviews of the chemistry of metals with special emphasis on biological effects have been prepared by Martell, Schubert and Nieboer (11–13), and two recent reviews (14,15) were devoted to the coordination chemistry of cadmium.

Chemistry of Cd

A chemical description of the Cd^{2+} ion, in relation to those metal ions known to interplay with Cd^{2+} in biological systems, i.e., Cu^{2+} and Zn^{2+}, as well as those donor groups available for complex formation in the biological systems, may contribute to the understanding of the ligand preference of Cd^{2+} during Cd intoxication, as well as the possibilities for induction of ligand exchange reactions during chelation therapy.

Together with Zn and Hg, Cd forms group IIB in the periodical table of the elements. Although a few compounds of Cd^+ exist, Cd is usually found in valence +2, with outer electron configuration $4d^{10}$. The polarizability of the Cd^{2+} ion is rather high (α = 1.8 Å³; 16), and coordination bonds formed with soft donors have a high degree of covalent character. Organic compounds of Cd (R_2Cd and RCd^+, R = aliphatic) exist (diethylcadmium is used in the production of tetraethyllead), but are unstable and decompose upon contact with water.

The stability of complexes formed between Cd and polar groups, in proteins (mainly those containing N, O or S), nucleic acids (the phosphate moieties or the bases) or added chelating agents, depend on energy changes associated with complex formation. The size of these energy changes depend on chemical features of the ligand (and the metal ion, in this case Cd^{2+}), the most important being stereochemical conditions at the ligand, "hardness/softness," the chelate effect and rate effects. These four factors of course interplay, and they cannot be considered independent of each other.

Stereochemistry of Cd^{2+} Complexes

Information about the stereochemistry of coordination complexes of Cd^{2+} with biological ligands in aqueous solution is of great importance for understanding the biological effects of Cd^{2+}. However, the largest part of information available concern the coordination chemistry of Cd^{2+} complexes in the solid state, examined by X-ray crystallographic methods. An extensive review of the stereochemistry of coordination compounds of Cd was recently published by Tuck (14). From the d^{10} state of the Cd^{2+} ion the coordination chemistry of Cd^{2+} would be expected to be dominated by examples of coordination to four donors of two electrons, according to the inert gas rule. Many examples of this are known. However, cadmium is also found in a large number of complexes with other coordination numbers ranging from 2 to 8, in many cases with the structure distorted from regular tetrahedral or octahedral. In Table 1 examples of coordination compounds of Cd^{2+} are given. It should be noted that, except for the aquocation of Cd^{2+}, the data pertain to nonaqueous conditions, and use of these data for extrapolation to biological systems is questionable.

The only known examples of two-coordinate Cd^{2+} species occur in gas phase. In aqueous phase such structures are hydrated. The $CdBr_3^-$ complex has pyramidal structure in aqueous solution, indicating hydration. The only known example of tetrahedral CdO_4 coordination is in crystalline $CdZn(PO_4)_2$. CdN_4 kernels have not been definately proven. However, a few examples of CdC_4 are known, and CdS_4 coordination is observed with several chelating agents with S-donor ligands, all with more or less distorted T_d structure. The stereochemistry of five-coordinated Cd^{2+} is varied, and many examples of distorted C_{4v} and D_{3h} symmetry have been reported. Examples of CdO_5, CdN_5 and CdS_5 coordination are known, in most cases forming dimers or polymers.

The aquo-cation of Cd^{2+} is $(Cd(H_2O)_6)^{2+}$. Several examples of CdO_6 and CdN_6 kernels have been reported. The highest number of S reported in hexacoordinate Cd^{2+} complexes is 4, in the kernels CdO_2S_4 and CdN_2S_4, and Cd^{2+} complexes containing S in the kernel with coordination numbers higher than six have not been reported.

Table 1. Stereochemistry of coordination complexes of Cd²⁺.

N^a	Compound	Coordin. Kernel	Stereochemistry	Reference
2	CdX_2(gas phase), X = F,Cl,Br,I	—	$D_{\infty h}$ symmetry	(17)
3	$CdBr_3^-$ (non aq. sol.)	—	D_{3h} symmetry	(18)
4	$CdZn_2(PO_4)_2$	CdO_4	Distorted T_d; polymeric lattice	(19)
	$Cd(CN)_2 \cdot en \cdot 2C_6H_6$	CdC_4	T_d; polymeric structure	(20)
	$Cd(2$-methyl thioxine$)_2$	CdN_2S_2	Distorted T_d; monomer	(21)
	$CdCl_2$thiourea$_2$	$CdCl_2S_2$	Distorted T_d; monomer	(22)
	$Cd(Et$-xanthate$)_2$	CdS_4	Distorted T_d; each S from different xanthate	(23)
5	$Cd_2Zn(PO_4)_2$	CdO_5	Trigonal pyramidal; polymeric lattice	(19)
	$Cd(N,N$-diethyldithio carbamic acid$)_2$	CdS_5	Distorted C_{4v}; dimer	(24)
	$Cd(thioglycollate)_2$	CdS_4O	Distorted D_{3h}; polymeric layer	(25)
6	$[Cd(OH_2)_6]^{2+}$ (aq.sol.)	CdO_6	O_h	(26)
	$Cd(acetylacetonate)_2$	CdO_6	Approx. O_h; parallel chain structures	(27)
	$[Cd(imidazole)_6]CO_3 \cdot 5H_2O$	CdN_6	Approx. O_h	(28)
	$Cd(NCS)_2$	CdN_2S_4	Approx. O_h; three-dimensional polymer, NCS-linked	(29)
	$Cd(HCOO)_2$(thiourea$)_2$	CdO_2S_4	Approx. O_h; chain polymer, S-bridged	(30)
	$Cd(L$-methionate$)_2$	CdO_4N_2	Distorted O_h	(31)
	$Cd(D$-penicillaminate$) \cdot H_2O$	CdO_3S_2N	Distorted O_h	(32)
7	$Cd(CH_3COO)_2 \cdot 2H_2O$	CdO_7	Square-based trigonal cap	(33)
	$Cd(malonate)_2 \cdot H_2O$	CdO_7	Distorted pentagonal bipyramid	(34)
8	$Cd(NO_3)_2 \cdot 4H_2O$	CdO_8	Bidentate NO_3 on each side of square planar $Cd(OH_2)_4$	(35)
	$Cd(maleate) \cdot 2H_2O$	CdO_8	Distorted dodecahedron	(36)

^aN = coordination number.

The stereochemistry of hexacoordinate Cd²⁺ is dominated by distorted or approximately O_h structure. Examples of heptacoordinate Cd²⁺ complexes with 4–7 O in the kernel are known, mainly with distorted pentagonal bipyramidal structure. In octacoordinate complexes, Cd²⁺ has been found in O_8 and N_8 kernels, mainly with distorted dodecahedron structure.

Scales for "Hardness/Softness"

The "hardness/softness" (or class A or B character) of metal ions, and thus their behavior (= ligand preference) in aqueous solution, is determined by physical and chemical characteristics of the ion. The same, of course, holds true for the donor groups. Some discrepancy exists in the chemical literature about whether to consider Cd²⁺ a "soft" ion or an "intermediate" ion. A little scrutiny on this subject may therefore be relevant.

Several authors have developed formal descriptors for tabulating donors and acceptors in complexation reactions into the three classes, "hard" or class A, "borderline" or "intermediate," and "soft" or class B. This classification was originally suggested by Schwarzenbach (37) and Ahrland et al. (38) and further elaborated by Pearson (39),

mainly on the basis of differences in the stability of metal halide complexes in water. Thus, a typical hard metal ion has the donor atom preference sequence: F⁻ > Cl⁻ > Br⁻ > I⁻, and a typical soft metal ion has the opposite preference sequence: I⁻ > Br⁻ > Cl⁻ > F⁻. Based on this definition, Cd²⁺ is a soft ion.

Several softness descriptors are listed in Table 2, and values for Cd²⁺ and other divalent ions tabulated: The ionic index correlates well with ionic interactions and indicates the propensity of the ion to form ionic bonds (40,41). The ratio of the sum of ionization energies of an ion over the ionic index, i.e., $r\Sigma I/Z^2$ was used by Williams as a measure of softness (41). Williams and Hale (41) defined the softness parameter Q as

$$Q = \frac{\Delta H_{ion(g)}(MF_n) - \Delta H_{ion(g)}(MI_n)}{\tfrac{1}{2}\,[\,(\Delta H_{ion(g)}(MF_n) + \Delta H_{ion(g)}(MI_n)\,]}$$

where $\Delta H_{ion(g)}(MF_n)$ is the coordinate bond energy, i.e., the enthalpy change for the reaction

$$MF_n \rightarrow M^{n+} + nF^-$$

in the gas phase, and analogously for iodide. Pearson and Mawbey (42) defined a related softness parameter

$$\sigma_p = \frac{\Delta H_{ion(g)}(MF_n) - \Delta H_{ion(g)}(MI_n)}{\Delta H_{ion(g)}(MF_n)}$$

The softness of metals increases with decreasing Q and decreasing σ_P.

Klopman (43) defined the softness parameter σ_K as the difference (in eV) between the empty frontier orbital energy and the desolvation energy of a metal ion. Ahrland (44) defined a softness parameter,

$$\sigma_A = (I_n + \Delta H_n)/Z$$

i.e., the difference between the total ionization potential and the dehydration energy $(-\Delta H_n)$ divided by the ion's charge. The σ_A increase with increasing softness.

The covalent index, $X_m^2 r$, where X_m is the electronegativity of the ions, was used by Nieboer and Richardson (13) to describe softness. The rationale is that X_m is related to the empty frontier orbital energy of a metal ion, and this parameter indicates the ability of the ion to accept electrons and form covalent bonds. $X_m^2 r$ increases with increasing softness.

In Table 2, several divalent metal ions are placed in order of average increasing softness, as based on the softness parameters. A reasonably good correlation between the different descriptors is demonstrated, although many different thermodynamic functions and experimental measurements have been used. The selection of exclusively divalent ions of course favors this agreement.

Generally, hard metal ions form stable complexes with hard donors, while soft metal ions form stable complexes with soft donors. However, softness itself favors formation of stable complexes. Thus, in a series of analogous coordination complexes between ions with the same charge and different ligands with the same number of donor groups, the stability of the complexes depends mainly on the softness of the ion and the ligand. This is illustrated in Table 3, which gives the stability constants of complexes between several divalent metal ions and different bidentate ligands. Table 3 demonstrates the general trend that the stability constants increase downwards and towards the right. This may be explained by increasing softness of the metals downwards and of the chelating agents' donor groups towards the right, i.e., the coordination bonds formed have a high degree of ionic character in the upper left part of the table and a high degree of covalent character in the lower right part. Table 4 summarizes the most important biological ligands, grouped according to their metal preference.

Thermodynamics of Complex Formation

The stability of coordination complexes can conveniently be described by thermodynamic functions. When n ligands form a coordination complex with a metal ion,

$$M + nA \rightarrow MA_n$$
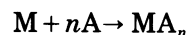
the ligational free energy, ΔG_n, is related to the stability constant:

$$\beta_n = (MA_n)/(M)(A)^n$$

through $\Delta G_n = -RT \ln \beta_n$. The energy change is contributed by changes in enthalpy, ΔH_n and entropy, ΔS_n:

$$\Delta G_n = \Delta H_n - T\Delta S_n$$

ΔH mainly describes energy changes due to bonding, while $T\Delta S$ mainly describes energy changes

Table 2. Values of softness parameters for divalent metal ions.

Hard-ness[a]	Metal ion	α	$\dfrac{Z^2}{r}$	$\dfrac{rI}{Z^2}$	Q	σ_P	σ_K	σ_A	$X_m^2 r$
H	Be^{2+}	0.01	9.09	3.02	0.20	0.172	−3.75	1.2	0.85
H	Mg^{2+}	0.10	4.87	—	0.21	0.167	−2.42	1.4	1.25
H	Ca^{2+}	1.10	4.04	4.45	0.21	0.180	−2.33	0.9	1.00
I	Ni^{2+}	1.00	7.79	3.30	—	0.126	−0.29	2.0	2.50
I	Fe^{2+}	1.20	5.40	4.45	—	0.127	−0.69	2.1	2.60
I	Co^{2+}	1.20	5.55	4.48	—	0.130	—	1.8	2.65
I	Mn^{2+}	1.25	5.00	4.61	—	0.124	—	2.0	2.00
I	Zn^{2+}	0.80	5.40	5.06	0.12	0.115	—	3.1	2.20
S	Cu^{2+}	—	5.55	5.04	—	0.104	0.55	3.1	2.95
S	Cd^{2+}	1.80	4.12	6.28	0.09	0.081	2.04	3.5	2.80
S	Pb^{2+}	4.80	3.33	—	0.14	0.131	—	4.1	3.40
S	Hg^{2+}	2.90	3.63	8.03	0.07	0.064	4.64	4.64	4.10
Reference		(16)	b	c	(41)	(45)	(45)	(45)	(13)

[a]H = hard; I = intermediate; S = soft.

[b]Calculated from the formal charge and the crystal ionic radius (46).

[c]Calculated from the ionic index and the total ionization potential (45).

Table 3. Equilibrium constants for complexes between divalent metals and simple bidentate ligands: M + L → ML.

Hard-ness[a]	Metal ion	log β				
		Oxalate	Glycine	Ethylene-diamine	Mercapto-acetate	Mercapto-ethylamine
H	Be2+	4.08[b]	—	—	—	—
H	Mg2+	2.76[b]	2.22[b]	0.37[c]	—	2.30[b]
H	Ca2+	3.00[c]	1.39[d]	—	—	2.21[b]
I	Ni2+	5.16[d]	5.78[b]	7.35[b]	(6.2)[f]	10.05[e]
I	Fe2+	3.05[d]	4.31[d]	4.34[c]	—	—
I	Co2+	3.84[b]	4.64[b]	5.96[c]	5.84[b]	7.68[e]
I	Mn2+	3.2[b]	2.80[b]	2.67[b]	4.38[b]	—
I	Zn2+	3.88[b]	4.96[b]	5.7[b]	7.86[b]	9.90[b]
S	Cu2+	4.84[b]	8.15[b]	10.54[b]	—	(16)
S	Cd2+	3.89[d]	4.22[b]	5.45[b]	—	10.97[b]
S	Pb2+	4.00[b]	5.47[d]	7.00[b]	(8.5)[f]	11.10[b]
S	Hg2+	9.66[b]	10.3[c]	14.3[b]	(24)[f]	—

[a]H = Hard, I = Intermediate, S = Soft. All values from Martell and Smith (47), except as noted.
[b]25°C, ionic strength 0.1.
[c]25°C, ionic strength 0.5.
[d]25°C.
[e]30°C, ionic strength, 1.0.
[f]Data of Jacobson and Turner (15).

related to the number of independent species, e.g., associated with changes in solvation. During complex formation with highly charged negative polydentate ligands such as the polyaminopolycarboxylates, the main contribution to the stability of the complex often is the increase in entropy due to loss of solvation, because of charge neutralization (Table 5). In the formation of complexes with uncharged ligands, e.g., the polyamines, the stability is generally due to decrease in enthalpy. The reason enthalpy changes are not totally outnumbered by entropy changes, e.g., during ligand selection in mixed chelation, is that the high dielectric constant of water efficiently screens electrostatic forces.

Chelating ligands form much more stable complexes than unidentate ligands: In series of analogous complexes with equal numbers of the same ligand, the stability generally increases with the number of rings formed. From the displacement reaction

$$MA_n + L_n \rightarrow ML_n + nA$$
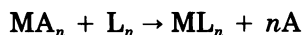
where I_n is a polydentate ligand with the same donor atoms as A, Schwarzenbach (49) defined the chelate effect as the logarithm to the equilibrium constant for the displacement reaction. This entity can be related to the change in free energy. Assuming (what may not always be true, however) that the change in enthalpy associated with complex formation is independent on whether the coordination groups are unidentate or bound together in a polydentate ligand, the chelate effect should be due to increased entropy. A significant enthalpy effect is, however, often observed, since

Table 4. Biological ligands.

Ligands preferred by hard ions	Ligands preferred by intermediate or soft ions	Ligands preferred by soft ions
OH^-	Cl^-	R-SH
H_2O	NH_3	R-S-R
CO_3^{2-}	N_2	R-S-S-R
SO_4^{2-}	O_2	
R-O-SO$_3^-$	$R-NH_2$	
NO_3^-	R_2NH	
HPO_4^{2-}	R_3N	
Mono- and diesters	R=N-R	
of phosphate	R-CO-N-R	
R-OH		
R-COOH		
R-CHO		
R-CO-R		
R-O-R		

Table 5. Effect of ring size on the chelate effect in the displacement reaction.

$Cd^{2+} + [CH_3\text{-}N(CH_2COO^-)_2]_2 \rightarrow$
$\qquad Cd^{2+} + [(^-OOCCH_2)_2N\text{-}(CH_2)_n\text{-}N(CH_2COO^-)_2]$

n	log β[a]	G, kcal/mole	H, kcal/mole	T/S
2	7.2	−5.3	−1.8	3.5
3	2.4	−1.8	1.8	3.6
4	−1	0.7	4.4	3.7
5	−1.6	1.2	2.8	1.6

[a]Calculated from ΔG, 20°C, 0.1 (48).

mutual repulsive forces between the donor groups are overcome by introducing them into one molecule. The chelate effect may be counteracted by adverse energy changes, due to necessary conformational changes during complex formation, especially when strained rings are formed. This is shown in Table 5: ΔH more than counteracts the entropy contribution to stability as the ring size increases.

Table 6. Stability of complexes between Cd^{2+} and polydental ligands, M + L → ML.

Ligand	Donor groups	log β
Malonic acid	2	2.64[a]
Citric acid	3	3.75[a]
Ditartronic acid	4	5.44[a]
Ethylenediamine (en)	2	5.45[a]
Diethylenetriamine (dien)	3	8.40[b]
Triethylenetetramine (trien)	4	10.63[a]
Tetraethylenepentamine (tetren)	5	14.00[a]
N,N,N',N-tetra(2-aminoethyl) ethylenediamine (penten)	6	16.10[b]
Iminodiacetic acid (IDA)	3	5.71[a]
Nitrilotriacetic acid (NTA)	4	9.78[a]
N-(2-Hydroxyethyl) ethylenediamine-N,N',N'-triacetic acid (HEDTA)	5	13.10[a]
Ethylenediaminetetraacetic acid (EDTA)	6	16.36[a]
Diethylenetrinitrilopentaacetic acid (DTPA)	8	19.00[a]

[a]25°C, 0.1.
[b]20°C, 0.1. Data from Martell and Smith (47).

At low concentrations of chelant, the chelate effect is an important contribution to stability. For the formation of a hexacoordinate Cd complex with a monodentate ligand Cd + 6A → CdA$_6$ the equilibrium constant is:

$$\beta_{(A_6)} = (CdA_6)/(Cd)(A)^6$$

At a decreasing concentration of ligand, the amount of complex will decrease with $(A)^6$, i.e., extremely fast, and for biological ligands (except water) $(A)^6$ is a very small number. For the formation of the analog complex with a hexadentate ligand containing 6A groups:
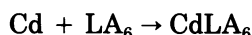
$$Cd + LA_6 → CdLA_6$$

the equilibrium constant is

$$\beta_{(LA_6)} = (CdLA_6)/(Cd)(LA_6)$$

and the amount of complex formed is directly proportional to the concentration of the ligand. Experimentally, the gain in stability due to chelate ring formation has been found to be in the order of 10^2 per ring formed, i.e., β_{LA_6} could be 10^{10} β_{A_6} or larger. The obvious consequence for chelation therapy during acute Cd intoxication is that at the nontoxic chelant concentrations the efficiency of chelation therapy can be improved most effectively by increasing the β value. This improvement can be obtained by increasing the number of chelate rings formed. According to Table 6, the introduction of one extra ring may increase β by a factor up to 10^4 (note that the complex MLA_n is compared with the complex MLA_{n+1}, thus only part of the increased stability is due to the chelate effect). The other way would be to increase the softness of the chelant's donor groups. According to Table 3, a change of "N" to "S" can increase β by approximately 4 orders of magnitude for complexes with soft metals. Table 7

Table 7. Stability of complexes between Cd^{2+} and substituted IDA.

Compound:	Equilibrium	Log β
Aliphatic $CH_3-N-(CH_2COO)_2$	ML/M·L	6.75[a]
	$ML^2/M·L^2$	12.43[a]
Alcohol $HO-CH_2-CH_2-N(CH_2COO)_2$	ML/M·L	7.24[a]
	$ML_2M·L^2$	12.31[a]
Ether $CH-O-CH_2-CH_2-N(CH_2COO)_2$	ML/M·L	7.53[b]
	$ML_2/M·L^2$	13.18[b]
Ketone $CH_3-CO-CH_2-N(CH_2COO)_2$	ML/M·L	7.37[a]
	$ML_2/M·L^2$	12.34[a]
Carboxyl $OOC-CH_2-N-(CH_2COO)_2$	ML/M·L	9.78[a]
	ML_2/ML^2	14.39[a]
Amine $H_2N-CH_2-CH_2-N(CH_2COO)_2$	ML/M·L	10.53[b]
	$ML_2/M·L^2$	16.59[b]
Mercapto $HS-CH_2-CH_2-N(CH_2COO)_2$	ML/M·L	16.72[b]
	$ML_2/M·L^2$	22.33[b]

[a]25°C, 0.1.
[b]20°C, 0.1. Data from Martell and Smith (47).

demonstrates that the stability of analogous complexes of Cd^{2+} with different compounds formed by substitution of the methyl group in N-methyliminodiacetic acid (MIDA) with donor groups of increasing softness, increases as expected: The change of –COOH to –CH_2SH yields an increase in stability of 7 to 8 orders of magnitude. It therefore seems a logical step to test chelants with as many SH groups as possible. The highest number of S atoms which can at the same time coordinate with Cd^{2+} seems to be 5. In hexacoordinate complexes, Cd^{2+} can coordinate with S_4N_2 and S_4O_2 (Table 1).

Effective SH-containing chelants for Cd^{2+} should have two vicinal SH groups (50). The tox-

Table 8. Rate constants for ligand exchange reactions.[a]

Metal ion	Ionic index	Exchange rate constant k_{M-H_2O}, sec^{-1}		Formation rate constant K_f, M^{-1}sec^{-1}	
		H$_2$O-H$_2$O	L-H$_2$O	NTA^{3-}	HEDTA^{3-}
Be^{2+}	9.09	2.1×10^3	10^2[b]	—	—
Mg^{2+}	4.87	5.3×10^5	3×10^5[b]	—	—
Ca^{2+}	4.04	4×10^8	$6-9 \times 10^8$[c]	—	$1-1.7 \times 10^7$
Ni^{2+}	7.79	3×10^4	$1-2 \times 10^4$[b]	4.8×10^5	1×10^6
Fe^{2+}	5.40	3.2×10^6	$1-2 \times 10^6$[b]	—	—
Co^{2+}	5.55	$1.1-2.4 \times 10^6$	$2 \times 10^5-2 \times 10^6$[b]	—	4×10^6
Mn^{2+}	5.00	3.1×10^7	$4 \times 10^6-5 \times 10^7$[b]	5×10^8	—
Zn^{2+}	5.40	3×10^7	3×10^7[b]	$2 \cdot 10^9-10^{10}$	$1-2 \times 10^9$
Cu^{2+}	5.55	5×10^9	$2-5 \times 10^8$[b]	2×10^8	8.4×10^7
Cd^{2+}	4.12	3×10^8	2.5×10^8[c]	$10^9-2 \times 10^{10}$	$2.6 \cdot 10^8-6.4 \times 10^9$
Pb^{2+}	3.33	—	7.5×10^9[c]	1.5×10^{11}	$23 \times 10^9-6 \times 10^{11}$
Hg^{2+}	3.63	2×10^9	2×10^9[d]	—	—

[a]Data from Margerum et al. (*52*).
[b]SO$_4^{2-}$.
[c]CH$_3$COO$^-$.
[d]Cl$^-$.

icity of propanetrithiol is much larger than that of BAL and related compounds, most likely due to adduct formation with proteins (*50*). Consequently, the toxicity may be reduced by introducing two pairs of vicinal SH groups, favouring tedrahedral coordination as in the Cd-MT complex. The efficiency of charged chelants as, e.g., the polyaminopolycarboxylic acids against the acute toxicity of parenterally administered Cd^{2+} decreases rapidly concomitantly with cellular uptake of Cd^{2+}, and not with the kinetics of MT induction (*51*). High efficiency of SH-containing chelants against aged Cd^{2+} deposits requires lipophilicity of the agent (*50*). Thus, the critical step in successful chelation of Cd^{2+}, except immediately after administration, seems to be transport over the cell membrane, first of the chelant and subsequently of the chelate.

By combining chemical considerations, such as those reviewed above with biological experiments it will hopefully be possible to design new and more effective chelating agents for Cd^{2+}, in order to antagonize the acute toxicity of Cd^{2+} as well as to detoxify aged Cd^{2+} deposits in the liver without inducing renal damage.

Rate Effects

The formation of coordination complexes in aqueous solution (in complex biological systems it could be called "ligand selection") is dependent on rate constants and equilibrium constants. Table 8 demonstrates a correlation between the aptitude of different ions for hydration and the ionic index, Z^2/r. The rate of exchange of water molecules from the inner hydration layer is largest for the less stable hydration complexes, and inspection of

Table 8 demonstrates that also the rate of simple displacement reactions, as expected, is inversely related to the ionic index. The ligand exchange rates for cadmium are generally large for water exchange, simple ligand exchange and complex formation with polydentate ligands (Table 8).

Thus, equilibrium will be reached rather fast, and after some time the distribution of Cd^{2+} between different ligands will reflect (1) the affinity of Cd^{2+} for the ligands as discussed above, i.e., mainly softness, and (2) stabilizing effects, i.e., mainly the chelate effect. Thus, during the initial phase of Cd^{2+} distribution in the body, equilibrium constants dominate, e.g., during complex formation with serum albumin in the blood stream, and subsequent ligand exchange from serum albumin to metallothionein (MT) in the liver. It has been demonstrated, however, that the process of *in vitro* chelation of Cd^{2+} out of the Cd-MT complex with EDTA is determined by the very slow rate of exchange, and not by the equilibrium (*53*). It is therefore of importance to consider rate constants for ligand exchange during the discussion of redistribution of Cd^{2+} after Cd^{2+} has been bound to MT.

Ligand exchanges are multistep reactions, and different mechanisms exist. The attacking ligand needs an initial coordination site on the complex. This can be achieved by solvent displacement or by displacing one of the coordination groups of the residential ligand. In the first case, the increased stability of the mixed complex will contribute to facilitate chelate ring opening. In the second case, the ring opening and subsequent displacement normally occur at a much slower rate. During the next step the attacking ligand must become chelated to decrease the rate of dissociation from the

mixed complex. Steric hindrance often decreases the exchange rate during reaction steps where several bulky groups simultaneously approach the metal ion. The kinetics of ligand exchange reactions have been extensively reviewed by Margerum et al. (52).

Effects of Competing Species on Chelate Stability

In biological systems, the conditional or effective stability constants β' for metal chelates may be several orders of magnitude lower than indicated by the standard stability constants, β shown in the tables, since competition by other ligands decreases the concentration of free metal ion, and competition by other cations, e.g., the H^+ ion and other metal ions, decreases the concentration of noncomplexed ligand available for chelation. The most important interfering ions in biological systems are normally the OH^- and H^+ ions ($\simeq 10^7$ M) and the Ca^{2+} ion ($\simeq 10^{-3}$ M). The term α is used for the ratio of the sum of concentrations of all species of the ion involved in complex formation with a competing ligand over the concentration of the free compound available for chelation, and analogous for competing ions: $\alpha_L(L) = \Sigma(L_i)$ and $\alpha_M(M) = \Sigma(M_j)$. For formation of the complex L_aM_b with one competing chelant and one competing ion present, the conditional stability constant, β' is:

$$\beta' = \frac{(L_aM_b)}{[\Sigma(L_i)]^a[\Sigma(M_j)]^b} = \frac{(L_aM_b)}{\alpha_L{}^a(L)^a\alpha_M{}^b(M)^b}$$

and

$$\beta' = \log \beta - a \log \alpha_L - b \log \alpha_M$$

Calculation of β' for chelation of Cd^{2+} with EDTA at pH = 7.4 with 2.5 mM Ca^{2+} present as in human serum (54) requires that four different equilibria be considered for the hydrogenated species of EDTA, giving

$$\alpha_{L/H} = \frac{(EDTA)+(HEDTA)+(H_2EDTA)+(H_3EDTA)+(H_4EDTA)}{(EDTA)}$$

$$= 1 + \beta_1(H) + \beta_2(H)^2 + \beta_3(H)^3 + \beta_4(H)^4$$

where the stability constants for the different hydrogenated forms of EDTA are: $\log \beta_1 = 10.17$, $\log \beta_2 = 6.11$, $\log \beta_3 = 2.68$, $\log \beta_4 = 2.0$ (47), and $\log \alpha_{L/H} = 2.77$. Four different hydroxyl complexes of Cd^{2+} should be considered, having $\beta_1 = 4.1$, $\beta_2 = 7.7$, $\beta_3 = 10.3$ and $\beta_4 = 12.0$ (47). The α value can be calculated analogously to the $\alpha_{L/H}$–value:

$$\alpha_{M/OH} =$$
$$1 + \beta_1(OH)^1 + \beta_2(OH)^2 + \beta_3(OH)^3 + \beta_4(OH)^4$$
with $\log \alpha_{M/OH} \simeq 0$.

Thus, competition by OH^- ions does not interfere with chelation reactions involving Cd at physiological pH.

There is only one EDTA species of Ca^{2+} competing for Cd, having $\beta = 10.61$ (47). We can now calculate $\alpha_{L/Ca} = 1 + \beta_1[Ca^{2+}]$ and by approximating $[Ca^{2+}] \simeq 2.5 \times 10^{-3}$ we find $\log \alpha_{L/Ca} = 7.01$.

For the CdEDTA complex, $\beta = 16.36$ (47). At the conditions specified the value of β' can be calculated to $16.36 - 2.77 - 7.01 = 6.58$ without taking into account mutual interactions between competing species: However, due to mutual interactions between ions the standard stability constants are not valid, and calculation of α-values in order to determine conditional stability constants becomes highly speculative. In serum also Mg^{2+} ($\simeq 0.86$ mM) (54), $Cu^{2+} + Cu^+$ ($\simeq 19$ μM) (54), and Zn^{2+} ($\simeq 46$ μM) (54) compete for Cd^{2+}. These metal ions are partially protein-bound, and exact knowledge about how available they are for competition is not present. Furthermore, chelation of Cd^{2+} with serum albumin during the initial phase after Cd^{2+} exposure and with metallothionein later will reduce the conditional stability constants for complexes of Cd^{2+} with chelating agents even more.

Binding of Cd^{2+} to Biological Ligands

In vivo, Cd^{2+} can bind to nucleic acids, or to proteins (Table 4). Several investigations have demonstrated that Cd^{2+} is not found associated with low molecular weight compounds like free amino acids, etc., to any appreciable extend, except maybe during a very brief period immediately after ingress of Cd^{2+}. Proteins offer much more favorable binding sites for Cd^{2+} than nucleic acids due to the soft binding sites formed by cystein residues.

In nucleic acids, the phosphodiester groups offer negatively charged coordination sites for hard ions like Mg^{2+} and Ca^{2+}, leading to bonding with mainly ionic character, while nitrogen-containing sites on the bases represent donor sites favored by Cu^{2+}, Cd^{2+} and Hg^{2+}. Intermediate ions like Co^{2+}, Mn^{2+} and Zn^{2+} can bind to either the phosphates or the bases. The deoxyribose moieties, however, are not suited for complex formation with metal ions.

A few investigations have yielded information about complex formation between Cd^{2+} and free

bases, nucleosides, and nucleotides. With cytidine monophosphate Cd^{2+} forms a complex, $[Cd(5'CMP)(H_2O)]_n$, in which $Cd^{2+}$is pentacoordinate, binding to phosphate groups from three different $5'$-CMP molecules, a water molecule and to N-3 of a fourth $5'$-CMP molecule (55). With $5' - $ AMP and NO_3^-, Cd^{2+} forms a complex $[5'AMP)Cd(NO_3)_2 \cdot H0]_2^{2+}$, in which two Cd^{2+} ions are octacoordinated and bind to N-3 and N-9 from two protonated adenine residues, arranged point symmetrically. Furthermore, the Cd^{2+}ions coordinate with the same two water molecules, and each Cd^{2+} ion coordinates with two of four NO_3^- ions. (15,56).

Less is known about binding of Cd^{2+} to DNA. In intact chromosomes from eukaryotic organisms, the DNA is present as a highly organized complex with a large number of different chromosomal proteins, histones as well as nonhistone proteins, the latter containing SH groups, which offer binding sites for Cd^{2+}. A very limited amount of information is available concerning interaction of Cd^{2+} with chromosomal proteins. In vitro exposure of human lymphocytes to Cd^{2+} can, however, inactivate the mitotic spindle (57,58), most likely by binding of Cd^{2+} to SH groups in spindle proteins. This inhibition of spindle function, which was also observed with Hg^{2+}, was counteracted by metallothionein induction after prolonged in vitro exposure to Cd^{2+}or Hg^{2+} (57,58). Sephadex G-75 chromatography of lysates of human lymphocyte cultures after 72 hr exposure to ^{109}Cd in vitro demonstrated binding of Cd to MT (Fig. 1).

The affinity of Cd^{2+} for proteins parallels to some degree the size of the stability constants for the complexes between the Cd^{2+} ion and the amino acids in the proteins. However, both the carboxyl groups and the amino groups are modified by peptide bond formation. Due to the uniformity of the peptide bond, functional side groups in the amino acids become major determinants of the affinity of metal ions for proteins. Table 9 shows the stability constants for the complexes of several amino acids with Cd^{2+}. Only histidine and cystein offer good binding sites for Cd^{2+} in proteins. Cd^{2+} can bind strongly to proteins if two SH groups are adjacent, allowing chelation. In metallothionein, Cd^{2+} is bound very strongly (log β = 25.5) (59). Optical studies (60) and ^{113}Cd–NMR studies (61) have indicated that in the metallothionein molecule Cd^{2+} is tetrahedrally coordinated to four deprotonated cysteinyl side groups by thiolate bonds, and that adjacent Cd^{2+} ions are linked through single thiolate bridges.

This strong binding of Cd^{2+} to MT is in sharp

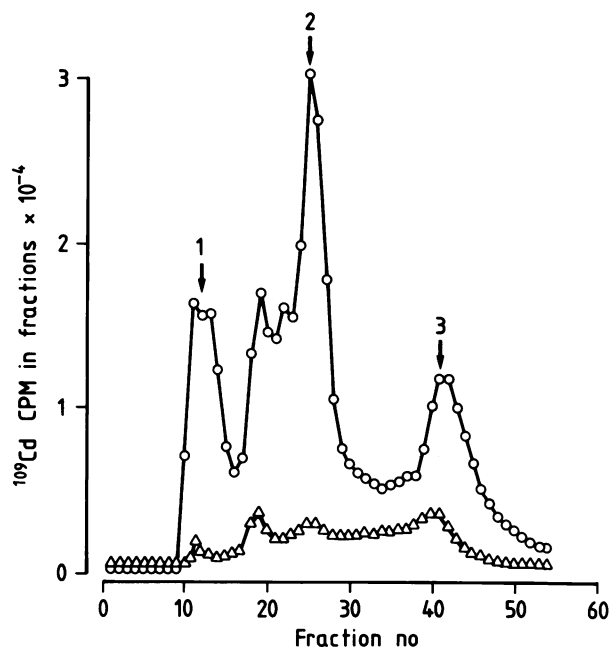FIGURE 1. Sephadex G-75 chromatograms of lymphocyte lysates after in vitro exposure to ^{109}Cd: ($\triangle$) 5×10^{-5} M Cd^{2+} 4 hr before harvest; (O) 5×10^{-5} M Cd^{2+} 72 hr before harvest; (1) elution volume of serum albumin; (2) elution volume of metallothionein; (3) elution volume of low molecular weight compounds.

Table 9. Stability of complexes between free amino acids and Cd^{2+}.[a]

Amino acid	ML/M·L log β_1	ML$_2$/M·L^2 log β_2
Alanine	3.80[b]	7.10[b]
Valine	3.46[b]	6.46[b]
Leucine	3.84[b]	6.54[b]
Isoleucine	7.00[b]	8.80[b]
Proline	—	(8.0)[d]
Phenylalanine	3.87[c]	6.73[c]
Tryptophane	4.47[c]	8.18[c]
Methionine	3.67[c]	7.03[c]
Glycine	4.22[c]	7.69[c]
Serine	(4.84)[d]	(7.4)[d]
Threonine	(4.43)[d]	(7.2)[d]
Cysteine	—	(9.9)[d]
Tyrosine	3.57[c]	6.08[c]
Asparagine	—	(7.1–8.6)[d]
Glutamine	—	(7.4)[d]
Aspartic acid	4.39[c]	7.55[c]
Glutamic acid	3.90[c]	(7.9)[d]
Lysine	—	(5.8)[d]
Arginine	—	(6.7)[d]
Histidine	5.39[c]	9.66[c]

[a]Values from Martell and Smith (47), except as noted.
[b]25°C, 0.5.
[c]25°C, 0.1.
[d]Data from Squibb et al. (62).

contrast with the kinetics of ligand exchange reactions involving Zn^{2+}. Removal of Zn from MT by EDTA during pseudo first-order kinetics for EDTA showed triphasic kinetics with k_1 fast, $k_2 \simeq 10^{-3}$ sec^{-1} and $K_3 \simeq 10^{-4}$ sec^{-1}, while the rate constant for removal of Cd^{2+} was approximately 3 $\times$ 10^{-6} sec^{-1} (53), and the rate constant for transfer of zinc to apo-carbonic anhydrase was found to be about 10^3, i.e., about the same rate as the reaction with free zinc, suggesting that a physiological role for MT could be acting as a zinc donor to zinc-requiring macromolecules (53). Due to the high degree of resistance of the Cd–MT complex against ligand exchange reactions, both favored by the large stability constant (59) and the sluggish reaction of the Cd–MT complex with competing ligands (53), Cd^{2+} is not likely to be removed from MT in vivo to any appreciable degree, except after translocation of the Cd–MT complex to the kidneys, where the MT moiety is broken down, and Cd^{2+} is rapidly reassociated with renal MT (63–65).

The efficiency of chelating agents for increasing renal excretion of Cd^{2+} during acute Cd^{2+} intoxication is sharply reduced concomitantly with cellular uptake of Cd^{2+}, mainly in the liver (51). Already 12 hr after administration of Cd^{2+}, only a limited amount of the dose is chelatable by antidotes like DTPA, EDTA and dimercaptosuccinic acid (66). It is, however, possible to chelate Cd^{2+} out of metallothionein and reduce the whole body burden as well as the concentration of Cd^{2+} in liver and kidneys by daily chelation therapy with BAL, initiated several days after cessation of Cd exposure (67). The main excretion route during BAL-induced mobilization of aged Cd deposits in the liver is fecal, due to increased biliary excretion (68).

Effects of Chelation on Cd^{2+} Toxicity

The possibilities of chelating agents for modifying the acute toxicity of Cd^{2+} depend on the chelating agent used, the molar ratio between the chelant and Cd^{2+}, the route of exposure and the time lapse between exposure to Cd^{2+} and to the chelating agent. Reported effects of chelating agents on acute Cd^{2+} toxicity include antagonistic effects, no appearant effect, and even enhancement of toxicity.

Parenteral Exposure

Nitrilotriacetic acid (NTA) increased the acute toxicity of Cd^{2+} in rats after subcutaneous expo-

sure (69). Several studies have demonstrated that subcutaneous exposure of mice to Cd^{2+} administered as a complex with NTA (70) or sodium tripolyphosphate (STPP) (70,71) increased the acute toxicity of Cd^{2+}, as indicated by mortality within 24 hr. The reason for the enhanced toxicity is probably a high hepatotoxicity of these complexes of Cd, since extensive hepatic necrosis was observed at doses of Cd^{2+} (3.2 or 3.35 mg/kg body weight), exerting mild or no hepatotoxicity when administered alone (70,71). The liver damage induced by the Cd–STPP complex was characterized by extensive centrilobular necrosis seen at 24 hr (71). During a time-course study of the induction of hepatic necrosis after subcutaneous administration of the STPP–Cd complex to mice, Andersen et al. (71) investigated livers of mice killed 2, 4, 6, 8, 12 and 24 hr after administration of 30 μmole/kg Cd with 90 μmole/kg STPP and observed pronounced centrilobular blood stasis and early centrilobular necroses with fragmentation and lysis of nuclei and swollen hepatocytes already 6 and 8 hr after administration. This picture progressed to a more advanced state at 12 hr, and already before 16 hr, 2/10 animals were dead. At 24 hr, 7/10 animals were dead. In animals given the same dose of Cd^{2+} without STPP the liver histology was normal during the first 12 hr. At 24 hr all animals had survived, and 4/8 animals had normal liver histology, while four livers showed necrosis of single or several hepatocytes with eosionophile, swollen cells and with admixture of neutrophiles. Focal necroses were randomly localized. All control animals had normal livers, and the kidneys of all animals appeared normal. The events inducing liver necrosis during Cd–STPP exposure must take place very rapidly after administration, since the minimum period required for induction of hepatic necrosis is 6–8 hr. The enhancement of toxicity exerted by STPP is most likely due to induction of increased rate of interorgan distribution of Cd^{2+} (71): Figure 2 demonstrates that both the transport away from the injection site and the accumulation of Cd^{2+} in liver and kidneys take place much faster when Cd^{2+} is administered as a complex with STPP than when Cd^{2+} is administered alone.

Investigation of the binding of Cd^{2+} to macromolecules by Sephadex G–75 chromatography demonstrated that STPP delayed and decreased the binding of Cd^{2+} to metallothionein in the liver (Fig. 3). Thus, soon after exposure, when metallothionein induction has not yet taken place, a high concentration of Cd^{2+} is present in the liver, able to exert a full toxic potential. The decrease in
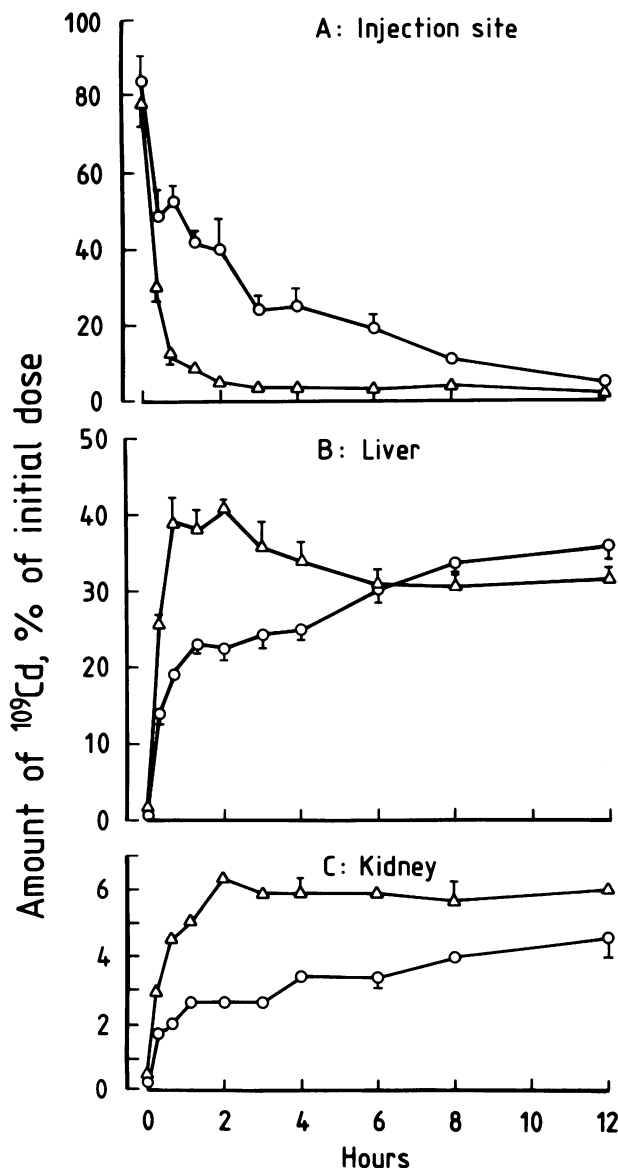
FIGURE 2. Effect of STPP on Cd transport and distribution among organs: (o) Cd, 30 μmole/kg; (Δ) Cd, 30 μmole/kg and STPP, 90 μmole/kg. Each point represents the average of determinations on four animals. The SEM is shown when larger than the height of the symbol.

FIGURE 3. Binding of Cd to proteins: (o) Cd, 30 μmole/kg; (Δ) Cd, 30 μmole/kg and STPP, 90 μmole/kg. From elution profiles like those shown in Fig. 4 the amount of ^{109}Cd bound to MT was calculated as % of total ^{109}Cd eluted from the column. The values obtained are shown versus time after injection.

the liver content of Cd^{2+} observed at later times most likely is due to leakage of Cd^{2+} from necrotic liver cells.

Sephadex G–75 chromatography (Fig. 4) of blood plasma, liver homogenate and kidney homogenate from animals killed 20 min after subcutaneous (SC) injection of Cd^{2+} or Cd^{2+} + STPP demonstrated that Cd in blood plasma was distributed in the MW area from serum albumin down to low molecular weight compounds. In the
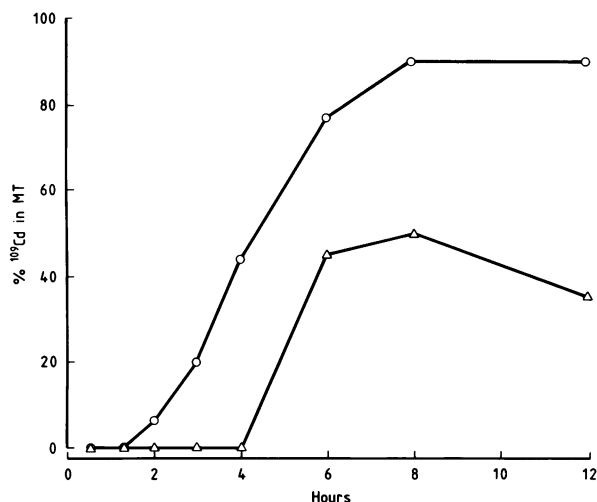
liver, Cd^{2+} was solely found associated with high molecular weight proteins (fractions 12–14), while in kidneys most of the Cd^{2+} was associated with high molecular weight compounds, but a small amount eluted in the MT region (fraction 23–25). In liver and kidneys Cd^{2+} was not found associated with low molecular weight compounds. There was no difference between the elution pattern between animals injected with Cd and Cd + STPP. One hour after injection, similar elution patterns were observed for liver and kidney homogenates, while all ^{109}Cd in plasma eluted with high molecular weight compounds, irrespectively of whether Cd or Cd + STPP had been injected. After Sephadex G–75 chromatography, ^{109}Cd and ^{109}Cd + STPP eluted in the low molecular weight area (≃ fraction 40). ^{109}Cd + STPP mixed with serum albumin eluted in the high molecular weight area. The unexpected elution pattern of Cd in plasma observed 20 min after injection has been verified in several trials. It may be due to splitting of high molecular weight complexes during chromatography, leading to extensive trailing.

In a study of the effect of cysteine on the renal uptake of Cd(72), SC coinjection of Cd^{2+} and cysteine decreased the liver concentration of Cd^{2+} and increased the kidney concentration of Cd^{2+} at 0.5–7 hr as well as 24 hr after injection, as compared with the same dose of Cd^{2+} alone. In contrast to the enhancing effect of NTA and STPP on acute Cd^{2+} toxicity, SC exposure of mice to Cd^{2+}
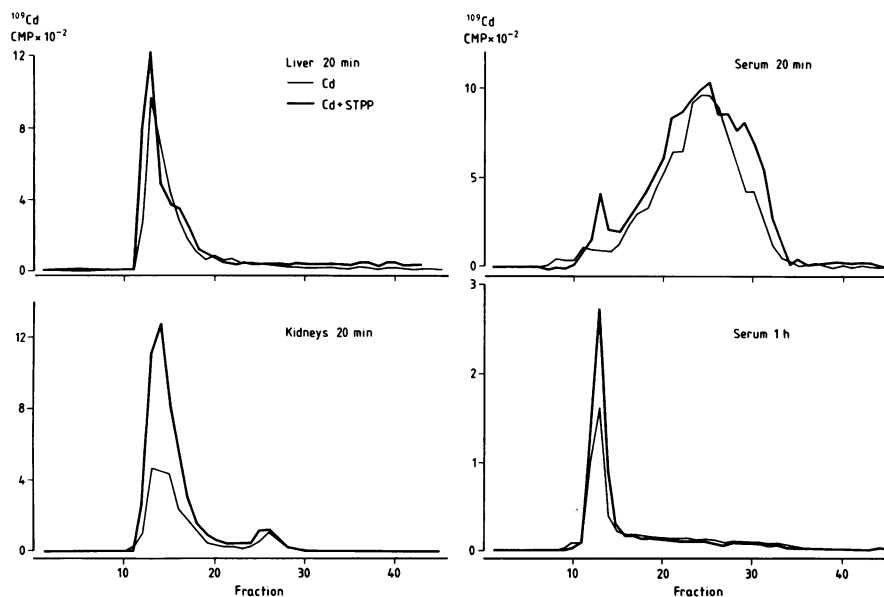
FIGURE 4. Distribution of [109]Cd among protein fraction after Sephadex G-75 gel chromatography. Same doses as in Figs. 2 and 3.

as a complex with EDTA reduced the acute toxicity, as well as the liver and kidney concentrations of Cd^{2+} after 21 days, both when the CdEDTA and when the Cd_2EDTA complex was dominating, as compared with the same dose of Cd^{2+} administered alone (73). Suzuki and Yamamura (74) injected rats intraperitoneally with Cd^{2+}, uncomplexed or complexed with chelating agents with different affinity for Cd^{2+}, and found after 4 days that the higher the stability constant of the complex with Cd^{2+}, the larger the ratio between the kidney and liver concentrations of Cd^{2+}, thus suggesting that the stability constant of the complex would determine the interorgan distribution.

Gastrointestinal Exposure

Eighteen months of chronic exposure of mice to Cd^{2+} (50 ppm in drinking water) administered as a complex with NTA (500 ppm), STPP (500 ppm) or EDTA (50 ppm) did not change the toxicity of Cd^{2+}, measured as mortality and pathological proteinuria, and only slight changes in the concentrations of Cd^{2+} in liver and kidneys were found, as compared with administration of Cd^{2+} alone (70). Chelation of Cd^{2+} with EDTA or BAL reduced the absorption of Cd^{2+} from ligated intestine after injection into duodenum in rats, as measured by [109]Cd uptake into intestinal tissue. In addition, the liver and blood concentrations of Cd^{2+} were decreased. BAL reduced the amount of

Cd^{2+} in the kidneys, while EDTA increased the amount of Cd^{2+} in kidneys, yielding a maximum at around 1.5 hr, while other much weaker chelating agents (cystein and gluthation) only marginally changed Cd^{2+} absorption (75).

The mortality in mice during 21 days, after administration of Cd^{2+}, either by stomach tube or by direct injection into the duodenum was significantly decreased when Cd^{2+} was given as the CdEDTA complex (Cd:EDTA = 1:4, molar ratio). However, when the predominating complex was Cd_2EDTA (Cd:EDTA = 1:0.4, molar ratio), the toxicity was identical to that exerted by the same dose of Cd^{2+} administered alone (73). The reduced toxicity of the CdEDTA complex was accompanied by a reduced liver and kidney burden of Cd. The reason the toxicity of the Cd_2EDTA complex approaches that of the Cd^{2+} ion is most likely that the Cd_2EDTA complex can split off one Cd^{2+} ion (73). The mortality during 3 weeks after a single dose of 60 mg/kg Cd^{2+} given by stomach tube was not changed when Cd^{2+} was administered as a complex with NTA (600 mg/kg) but was totally abolished when Cd^{2+} was complexed with STPP (600 mg/kg) (76).

Translocation of Cd^{2+} from Liver to Kidneys

In experimental animals, the liver has been found to contain the largest amount of Cd^{2+} dur-

ing chronic Cd^{2+} exposure although the kidneys after prolonged exposure eventually may contain the largest concentration of Cd^{2+} (77,78). The Cd^{2+} deposit in kidneys is stable and urinary Cd^{2+} excretion is low before tubular damage has been induced (79,80). Thus, Cd^{2+} concentrations of the order of 200–300 mg/kg in kidneys may be reached, and such concentrations have been suggested as the critical concentration for induction of kidney dysfunction (6,81–84). After induction of tubular damage, urinary Cd^{2+} rises sharply (85), and Cd^{2+} bound to MT is excreted into the urine (86,87). During the period before induction of tubular damage the interorgan distribution of Cd^{2+} is mainly due to slow translocation of Cd–MT (and the small amount of non–MT bound Cd^{2+}) from the liver to the kidneys (78). The retention half-life of a single dose of Cd^{2+} in rats has been measured to be 73 days in the liver and at least 1240 days in the kidneys (88). Most likely this translocation involves transport of liver Cd–MT complex, because circulating Cd–MT complex has been demonstrated in the plasma of mice and rats after prolonged Cd^{2+} exposure (86,87,89–94), and MT has been demonstrated in the plasma of rats already 1 day after a single dose of Cd^{2+}, by the use of a radioimmunoassay (95). MT has also been demonstrated in plasma and urine of Cd exposed humans (94,96–100).

Parenterally administered Cd–MT complex is preferentially absorbed by renal tubules (101–104) and exert a much higher renal toxicity than Cd^{2+} administered alone (79,103,105–107). After glomerular filtration and tubular resorption, the Cd–MT complex is rapidly degraded, and Cd^{2+} is re-incorporated into renal MT (63–65,108). During the period of slow translocation of Cd^{2+} from liver to kidneys, biliary excretion seems to contribute considerably to Cd^{2+} detoxification (109).

Nordberg et al. (110) concluded that, after long-term Cd^{2+} exposure, the largest amount of soluble Cd^{2+} in mouse blood cell hemolysate was Cd–MT complex in erythrocytes. In vitro exposure of human lymphocytes to $^{109}Cd^{2+}$ showed that induction of MT synthesis and Cd–MT complex formation occur in lymphocyte cytoplasm (57,111). Quantitation of Cd^{2+} in plasma as well as in red and white blood cells, 60 hr after a single dose of Cd^{2+} to rats, showed that, although the highest concentration of Cd^{2+} was found in white blood cells, the most important contribution to the blood content of Cd^{2+} was Cd^{2+} in erythrocytes, mainly bound to cell membranes. Cd^{+} in the erythrocyte cytosol was partly bound to high molecular weight proteins, partly to a protein with MW like MT (112).

Table 10. Effect of dietary PCB exposure on Cd transition from liver to kidneys.

Expt. No.	PCP in diet, ppm	Group	^{109}Cd, % of initial dose ± SD (p)[a]	
			Liver	Kidneys
1[b]	0	1 (n=4)	50.27 ± 1.72	8.7 ± 1.10
			—	—
	10	2 (n=5)	47.42 ± 3.84 (ns)	10.80 ± 0.92 (p<0.005)
	25	3 (n=6)	50.14 ± 3.59 (ns)	9.15 ± 0.65 (ns)
	50	4 (n=8)	49.18 ± 0.93 (ns)	11.30 ± 1.77 (p=0.01)
	100	5 (n=5)	48 ± 2.33 (p<0.025)	11.31 ± 1.49 (p<0.0025)
	200	6 (n=8)	46.36 ± 2.36 (p<0.01)	11.66 ± 1.21 (p<0.0025)
2[c]	0	7 (n=6)	17.74 ± 4.03	29.76 ± 4.0
			—	—
	10	8 (n=5)	15.43 ± 2.72 (ns)	26.61 ± 3.63 (ns)
	25	9 (n=5)	14.25 ± 4.53 (ns)	27.28 ± 1.22 (ns)
	50	10 (n=5)	10.53 ± 2.65 (p<0.0025)	26.82 ± 5.25 (ns)
	100	11 (n=7)	11.42 ± 4.52 (p<0.05)	30.54 ± 3.84 (ns)
	200	12 (n=5)	9.02 ± 2.46 (p<0.0005)	32.48 ± 3.49 (ns)

[a]Student's t-test, two-sided.
[b]^{109}Cd dose, SC; 10 μmole/kg; 10 μCi/animal; animals killed after 15 days.
[c]^{109}Cd dose, SC; 0.1 μmole/kg; 1 μCi/animal; animals killed after 15 days.

Effect of Hepatic Damage on Cd^{2+} Metabolism

Due to the large deposit of Cd^{2+} present in the liver during Cd^{2+} exposure, it is likely that hepatic disorders may alter the tissue distribution of Cd^{2+}. This has been verified in a few investigations where acute liver damage resulted in rapid and substantial translocation of aged Cd^{2+} deposits from liver to kidneys. Induction of hepatic necrosis by aflatoxin B_1 lead to translocation of Cd^{2+} administered 1 month earlier from liver to kidneys. Approximately 40% of the decreased liver burden of Cd^{2+} was recovered as increased kidney burden, most likely transferred as the MT–Cd complex (113). Intragastric administration of 1 mL/kg CCl_4 to rats at least 2 weeks after the end of exposure to 200 pm Cd^{2+} in the drinking water for 1 or 4 months also induced a decrease in the liver content of Cd^{2+}, paralleled with recovery of some of the Cd^{2+} in the kidneys. In this study a strongly increased urinary excretion of Cd^{2+} as well as proteinuria was also in-

duced by CCl_4 (114). The proteinuria was largest in the group of rats exposed to Cd^{2+} for 4 months.

In a similar study, aged Cd^{2+} deposits in the liver of rats were also mobilized by hepatic necrosis induced by 2.5 mL/kg of CCl_4 administered 1 day after cessation of 1–8 weeks exposure to repeated SC injections of Cd^{2+}. The hepatic concentration of Cd^{2+} was almost reduced by 50% 24 hr after CCl_4 exposure. Highly increased plasma and urinary concentrations of Cd^{2+} were found, and Sephadex G–75 chromatography indicated that the Cd–MT complex accounted for most of the Cd^{2+} present in plasma and urine. The kidney concentration of Cd^{2+} was also increased. Only a small part of the mobilized liver Cd^{2+} content was, however, recovered in kidneys. The Cd^{2+} content in other organs (spleen, small intestine, pancreas) and in feces was not increased, compared with control animals (115,116). Since CCl_4 is nephrotoxic and induces proximal tubular damage (117), evaluation of these studies in relation to the toxicity of Cd–MT to the proximal renal tubule is difficult.

Little is known about effects of chronic liver damage on Cd^{2+} translocation. In an unpublished study, Andersen et al. (118) found that the rate of translocation of Cd^{2+} from liver to kidneys was increased in mice with hepatic necrosis induced by chronic PCB exposure. Mice fed diets containing 0, 10, 25, 50, 100 or 200 ppm PCB were given a single SC dose of ^{109}Cd (see Table 10). Dietary PCB exposure for 36 weeks induced dose-dependent pathological changes. In the group fed 200 ppm PCB centrilobular liver cell enlargement with increased diameter of both cytoplasm and nuclei was seen, as well as focal necrosis with Kupffer cell proliferation. Some confluent necroses, a few macroscopically visible, were also observed. In mice killed 2 weeks after a single SC dose of $^{109}Cd^{2+}$, slightly lower amounts of Cd^{2+} were present in the liver and slightly larger amounts were found in kidneys in animals exposed to 100 and 200 ppm PCB, as compared to control animals. Fifteen weeks after SC $^{109}Cd^{2+}$ exposure, a dose-dependent, highly significant reduction in hepatic Cd^{2+} content was observed; this decrease was, however, not followed by a comparable increase in the kidney content of Cd^{2+} (Table 10). Thus, during the presumably very slightly increased rate of translocation of Cd^{2+} due to PCB–induced chronic liver damage, some of the mobilized Cd^{2+} seem to be excreted, probably in the urine. Whole body counting of the animals could not demonstrate PCB-induced changes in the rate of excretion of SC dose of $^{109}Cd^{2+}$ during 15 weeks. The difference in liver +

kidney burden of Cd^{2+} between the control group and the group exposed to 200 ppm PCB at 15 weeks is, however, only about 6% of the initial dose (Table 10), thereby explaining why a possible slight increase in detoxification rate was not observed.

So far the effect of acute or chronic liver damage on the renal toxicity of chronic low level Cd^{2+} exposure is virtually unknown. The studies reviewed here indicate that hepatic Cd^{2+} mobilized due to acute or chronic liver damage is not quantitatively recovered in the kidneys. The possibility does, however, exist that humans with liver damage, e.g., due to excessive alcohol consumption have an increased risk for tubular kidney damage if they are exposed to Cd^{2+}. Furthermore, people with a high liver burden of Cd^{2+} due to former or present Cd^{2+} exposure may be at risk for kidney damage if acute liver damage occurs, e.g., due to hepatitis. Further experimental investigation of this aspect of Cd^{2+} toxicodynamics is needed.

Chelation Therapy

According to recent reviews (51,119,120) only a limited knowledge is presently available concerning the possibility for chelate treatment of Cd^{2+} intoxication in humans. Although BAL and EDTA increase the renal excretion of Cd^{2+} in experimental animals, both antidotes have been reported also to increase the renal injury produced by Cd^{2+} (121,122). Intravenous administration of $CaNa_2$-EDTA (which is superior to the sodium salt of EDTA, which may induce hypocalcemic tetany as a complicating side effect) increases the renal excretion of zinc considerably, and the excretion of iron and copper slightly (119). Extensive destruction of proximal tubular epithelium has been observed in the kidneys of humans and experimental animals after EDTA treatment (123–125). Due to the mobilizing effect on aged Cd^{2+} deposits, chelation therapy of acute Cd^{2+} intoxication is contraindicated in humans with any significant previous Cd^{2+} exposure (6,119).

In order to maximize the efficiency of chelation therapy against a toxic metal ion and to minimize the unwanted excretion of essential metal ions (i.e., to obtain a high value of β', the conditional stability constant for the complex between the toxic ion and the selected chelating antidote), knowledge about the stabilities of complexes with competing metal ions is necessary. In Table 11 the stability constants for complexes between the most frequently used antidotes and competing

Table 11. Stabilities of complexes between important chelating agents and Cd^{2+} or other biologically important ions: $M + L \rightarrow ML$.[a]

Compound	log β				
	Ca^{2+}	Mg^{2+}	Cu^{2+}	Zn^{2+}	Cd^{2+}
Sodium tripolyphosphate (STPP)	5.20	5.76	8.3	7.5	6.58
2,3-Dimercaptopropanol (BAL)	—	—	—	13.5	—
Penicillamine (PEN)[b]	—	—	19.5	9.40	10.8
Nitrilotriacetic acid (NTA)	6.39	5.47	12.94	10.66	9.78
Ethylenediaminetetraacetic acid (EDTA)	10.61	8.83	18.70	16.44	16.36
Diethylenetrinitrilotetraacetic acid (DTPA)	10.57	9.34	21.38	18.29	19.0
Ethylene bis(oxyethylenenitrilo)tetraacetic acid (EGTA)	10.86	5.28	17.57	12.6	16.5

[a]Data from Martell and Smith, 25°C, 0.1, except as noted.
[b]20°C, 0.1.

metal ions are listed. DTPA, which has proven superior as an antagonist toward the acute toxicity of Cd^{2+} (126,127), forms a complex with Cd^{2+} several orders of magnitude more stable than EDTA, while STPP and NTA, which in fact increase the acute toxicity of parenterally administered Cd^{2+} (69–71) and also PEN, which is without beneficial effects on acute Cd^{2+} toxicity (126,127), form much weaker complexes with Cd^{2+}.

As indicated above, mobilization of aged Cd^{2+} deposits in the liver by chelating agents may increase the risk for development of tubular damage. Cherian and co-workers (50,68) and Von Burg and Smith (128) have demonstrated that renal excretion of Cd^{2+} can be circumvented by the use of BAL or structurally related di- and trimercaptans, which increase the biliary excretion of Cd^{2+}. The requirement for induction of efficient detoxification of the Cd-MT complex in liver seems to be a relatively lipophilic chelating agent with two adjacent SH groups. It is presently not known whether these interesting results pertain to the human situation, since the high toxicity of this kind of compound in laboratory animals seems to contraindicate extensive human use.

Most published experiments evaluating antagonizing effects of chelating agents on acute Cd^{2+} toxicity have been performed by administration of Cd^{2+} to experimental animals by the intravenous or other parenteral routes. Accidental acute Cd^{2+} intoxication in humans is, however, always due to either gastrointestinal or pulmonary exposure. Effects of chelating agents on acute Cd^{2+} toxicity after administration via these routes are very poorly investigated, and a very limited knowledge is available.

Future experiments investigating effects of chelating agents on acute Cd^{2+} toxicity, biliary and urinary excretion and organ distribution of Cd^{2+} after administration via these routes are of great importance for developing chelation ther-

apy regimens applicable to treatment of acute human Cd^{2+} poisoning.

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